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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat [OPPTS 870.3700 (§83-3)]

DP BARCODE: D270629 SUBMISSION CODE: S587122
P.C. CODE: 098951

TEST MATERIAL (PURITY): 1,2-Benzisothiazolin-3-one, 2-butyl- (95.5% a.i.)

SYNONYMS: Substance S123386

CITATION: Moxon, M.E. (1997) Substance S123386: Developmental Toxicity Study in the Rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No. CTL/P/5328. Laboratory Study No. RR0714. January 23, 1997. MRID 44364920. Unpublished.

SPONSOR: Zeneca Biocides, Wilmington, Delaware 19850-5457

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44364920), 24 presumed pregnant Alpk:AP$_f$SD (Wistar derived) rats per group were administered 1,2-Benzisothiazolin-3-one, 2-butyl-(95.5%; Batch No. JM 5420/80) by gavage in corn oil at doses of 0, 30, 100, or 300 mg/kg/day on gestation days (GD) 7-16, inclusive. All doses were adjusted for purity. On GD 22, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally, viscerally, and skeletally. Heads from all fetuses were cut along the fronto-parietal suture line and the brain was examined.

No treatment-related deaths occurred during the study but several intercurrent deaths due to gavage error were observed. All remaining animals survived to scheduled sacrifice. Treatment-related clinical signs of toxicity among surviving animals were limited to abnormal respiratory noise in 4/24 high-dose dams on GD 8-19 compared with none in the controls or other treated groups.

Body weights, body weight gains, and food consumption by the low- and mid-dose groups were similar to the controls throughout the study. Adjusted (based on GD 7) body weights of the high-

dose group were significantly ($p \leq 0.05$ or 0.01; 96-99% of controls) less than that of the controls from GD 8 through GD 19. Body weight gain by the high-dose group was 82% of the control group level during the dosing interval and reflects slightly (n.s.) lower gravid uterine weights for these dams. Food consumption by the high-dose group was 83-86% ($p \leq 0.01$) of that of the controls during the treatment interval.

At the scheduled gross necropsy, ulcerated areas of the stomach were observed in 0, 1, 5, and 16 animals in the control, low-, mid-, and high-dose groups, respectively. In addition, the mid-dose animal which died also had ulcerated areas of the stomach. These lesions were considered to be indicative of local irritation from the test compound. No other treatment-related lesions were observed in any animal.

Therefore, the maternal toxicity LOAEL for the test compound in rats is 100 mg/kg/day based on ulcerated areas of the stomach. The maternal toxicity NOAEL is 30 mg/kg/day.

No treatment-related differences were observed between the treated and control groups for number of corpora lutea, numbers of implantation and resorption sites, pre- or post-implantation losses, gravid uterine weights, fetal body weights, number of fetuses/litter, or fetal sex ratios. No surviving dam had complete litter resorption.

The total number of fetuses(litters) examined for external, visceral, and skeletal malformations/ variations was 346(24), 272(22), 273(22), and 298(23) in the 0, 30, 100, and 300 mg/kg/day groups, respectively. No treatment-related external, visceral, or skeletal malformations/ variations were observed in any group.

Therefore, the developmental toxicity NOAEL for the test compound in rats is ≥300(HDT) mg/kg/day and the developmental toxicity LOAEL is not identified.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3)] in rats.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Flagging, and Data Confidentiality claim statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 1,2-Benzisothiazolin-3-one, 2-butyl-

Description:	dark brown liquid
Batch No.:	JM 5420/80
Purity:	95.5% a.i.

Stability of compound: used within the stated expiry date
Storage Conditions: Ambient temperature in the dark

2. Vehicle and/or positive control

Corn oil (CTL reference Y00790/007) was used as the vehicle and negative control.

3. Test animals

Species: rat
Strain: Alpk:AP,SD (Wistar derived)
Age and weight at study initiation: 10-12 weeks; 213-301 g
Acclimation period: none
Source: Rodent Breeding Unit, Alderley Park
Housing: Animals were individually housed.
Diet: CT1 diet was available *ad libitum*.
Water: Public drinking water supply was available *ad libitum*.
Environmental conditions:
Temperature: 21±2°C
Humidity: 40-70%
Air changes: 25-30/hour
Photoperiod: 12 hr light/dark

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Substance S123386 when administered by gavage to rats on GD 7-16, inclusive.

1. In life dates

Start: August 20, 1996; end: September 20, 1996

2. Mating

Females were paired overnight to a male of the same strain while at the breeding facility. The day when spermatozoa were detected in a vaginal smear was designated as GD 1 and on this same day successfully mated females were shipped to the Central Toxicology Laboratory testing facility.

3. Animal assignment and dose selection are presented in Table 1. The study was divided into 24 replicates each containing one cage per group. Computer-generated, random number permutations were used to allocate the cages within each replicate to an experimental group. On arrival at the testing facility, each rat was randomly allocated to a cage. Females mated to the same male were distributed across groups.

TABLE 1. Animal assignment*		
Test Group	**Dose Level (mg/kg/day)**	**Number Assigned/Group**
Control	0	24
Low Dose	30	24
Mid Dose	100	24
High Dose	300	24

*Data taken from text table p. 16, MRID 44364920.

4. Dose selection rationale

Doses were selected on the basis of range-finding studies in the pregnant rat conducted by the testing laboratory. Details of these studies were not included in the current report.

5. Dose solution preparation and analysis

Test solutions were prepared once prior to study initiation and stored at room temperature. Each solution was prepared by adding a weighed amount of test article (adjusted for purity) to an appropriate amount of vehicle. Each solution was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used each day. Dose solutions were shaken prior to dosing and during dosing as required. Concentration and homogeneity of the dosing solutions were determined from samples taken from each solution prior to study initiation. The sampling points for homogeneity determination were labeled as "start", "middle", and "end". Stability was determined by reanalysis of the low- and high-dose solutions after 27 days.

Results -

Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the dose solutions were 99.7-100.0% of nominal.
Homogeneity analysis: Mean concentrations of the three samples from each dosing solution differed by <4%.
Stability analysis: Samples measured after storage for 27 days were 98-99% of their initial measured concentrations.

Analyses of the dosing solutions indicated that the test article was adequately mixed in the vehicle and stable for the duration of use and that actual doses to the animals were acceptable.

6. Dosing

All doses were administered in a volume of 1 mL/100 g of body weight based on daily individual body weights.

C. OBSERVATIONS

1. Maternal observations and evaluations

Detailed clinical observations were recorded daily. Cage-side observations were also made as soon as possible after dosing and daily towards the end of the working day. Body weights were measured on GD 4, prior to dosing on GDs 7-16, and on GDs 19 and 22. Food consumption was measured for the intervals of GD 1-4, 4-7, 7-10, 10-13, 13-16, 16-19, and 19-22. Dams were sacrificed on GD 22 by overexposure to halothane vapor and examined grossly. The number of corpora lutea on each ovary were counted. Gravid uteri were weighed and examined for number and location of live and dead fetuses and number and location of early and late resorptions and implantation sites. For animals that appeared nongravid, the uterus was stained with ammonium polysulphide for visualization of implantation sites.

2. Fetal evaluations

At necropsy, each live fetus was weighed, killed with an intracardiac injection of pentobarbitone, and examined for external abnormalities. Each fetus was examined viscerally by fresh dissection, sexed, eviscerated, and fixed in 70% industrial methylated spirits. After approximately 24 hours, the head of each fetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. All carcasses were processed and stained for skeletal examination including assessment of ossification of the *manus* (hand) and *pes* (foot).

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weight data were analyzed by Analysis of Covariance (ANCOVA) based on GD 7 body weight. Maternal food consumption data, numbers of implantations and viable fetuses, fetal body weight data, gravid uterine weights, litter weight, and *manus* and *pes* scores were analyzed by Analysis of Variance (ANOVA). Proportion data were analyzed with Fisher's Exact test and percentages were analyzed by ANOVA following double arcsine transformation of Freeman and Tukey.

2. Historical control data were provided to allow comparison with concurrent control and treated groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

One low-dose animal was found dead on GD 17, one mid-dose animal was found dead on GD 16, and one high-dose animal was sacrificed moribund on GD 8. Gavage error was confirmed at necropsy of all three of these animals. All remaining animals survived to scheduled sacrifice. Treatment-related clinical signs of toxicity among surviving animals were limited to abnormal respiratory noise in 4/24 high-dose dams on GD 8-19 compared with none of the controls or other treated animals. No gross necropsy findings were noted in these animals. Other clinical signs were either limited to a single animal per group, occurred equally in the control and treated groups, or the incidences were not dose-related.

2. Body weight

Selected maternal body weights and body weight gains during gestation are given in Table 2. When adjusted for GD 7 body weight, weights of the high-dose group were significantly ($p \leq 0.05$ or 0.01) less than that of the controls from GD 8 through GD 19. However, body weights of the high-dose group were only 1-4% less than the controls. Body weight gain by the high-dose group was 82% of the control group level during the dosing interval. Body weights and body weight gains by the low- and mid-dose groups were similar to the controls throughout the study.

TABLE 2: Maternal body weights and body weight gains during gestation (g)*				
GD	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
1	252.1	251.0	254.6	251.8
7	285.3	285.0	288.2	284.3
8[a]	288.7	288.9	288.4	284.8** (99)[b]
10	299.2	300.6	298.0	295.1* (99)
12	310.1	312.0	309.9	304.5** (98)
14	323.2	324.3	322.2	315.6** (98)
16	337.9	338.9	338.5	328.5** (97)
19	376.7	376.5	375.1	362.3** (96)
22	409.6	410.0	412.4	401.1
Body weight gain days 1-7[c]	33.2	34.0	33.6	32.5
Body weight gain days 7-16 (dosing interval)[c]	52.2	53.2	53.3	42.7 (82)

*Data taken from Table 6, pp. 35-36, MRID 44364920.

[a]Body weights GD 8-22 adjusted for GD 7 weights.

[b]Number in parentheses is percent of control; calculated by reviewer.

[c]Calculated by reviewer from unadjusted group means.

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. Food consumption

Food consumption by the high-dose group was 83-86% ($p \leq 0.01$) of that of the controls during the treatment interval. During the post-dosing interval, food consumption by the high-dose group was significantly ($p \leq 0.05$; 92% of controls) less than that of the controls for GD 16-19 but recovered to the control level by GD 19-22. Food consumption by the low- and mid-dose groups was similar to the controls throughout gestation.

4. Gross pathology

At scheduled necropsy, ulcerated areas of the stomach were observed in 0, 1, 5, and 16 animals in the control, low-, mid-, and high-dose groups, respectively. In addition, the mid-dose animal found dead on GD 16 also had ulcerated areas of the stomach. No other treatment-related lesions were observed in any animal.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 3. For all treated groups, gravid uterine weights and numbers of fetuses/litter were decreased, pre- and post-

implantation losses were increased, and early resorptions were increased as compared with the control values. Also, fetal body weights in the treated groups were greater than that of the control. Statistical significance was attained by one or two treated groups for some of these parameters but a dose-related response was not observed for any endpoint.

TABLE 3: Cesarean section observations*

Observation	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
No. Animals Assigned	24	24	24	24
No. Animals Pregnant	24	23	23	24
Pregnancy Rate (%)	100	96	96	100
Maternal Mortality	0	1	1	1
Delivered Early/Aborted	0	0	0	0
Mean Gravid Uterine Wt (g)	98.1	86.0*	88.4	89.5
Corpora Lutea/Dam	16.0	16.4	15.7	15.6
Implantation/Dam	14.8	13.5	13.5	14.0
Preimplantation Loss (mean %)	7.0	17.6*	14.3	10.6
Postimplantation Loss (mean %)	3.2	8.2	8.1	7.4
Total Live Fetuses	346	272	272	297
Live Fetuses/Litter	14.4	12.4*	12.4*	13.0
Mean Fetal Weight (g)	4.88	4.97	5.07*	5.00
Sex Ratio (% Male)	52.6	49.1	55.0	53.0
Dams With All Resorptions	0	1[a]	1[a]	0
Resorptions/Dam				
Early Resorptions (%)	2.9	6.9	6.6	7.0*
Late Resorptions (%)	0.3	1.2	1.5	0.4

*Data taken from Tables 4 and 9, pp. 32 and 40-41, respectively, MRID 44364920.

[a]Intercurrent death.

Significantly different from control: *$p \leq 0.05$.

B. DEVELOPMENTAL TOXICITY

The total number of fetuses(litters) examined for external, visceral, and skeletal malformations/variations was 346(24), 272(22), 273(22), and 298(23) in the 0, 30, 100, and 300 mg/kg/day groups, respectively. No treatment-related fetal anomalies were observed in any group. A summary of the fetal findings is given in Table 4.

1. External examination

No treatment-related external malformations were observed in any fetus. A high-dose fetus had gross malformation of the body which was not further defined. In the mid-dose group, one fetus had bilateral microphthalmia and abnormal pinna and another from a different litter had left microphthalmia. No external variations were observed.

2. Visceral examination

One control fetus and two mid-dose fetuses from different litters had hydrocephaly. One of the mid-dose fetuses also had multiple malformations. Dilated and/or kinked ureter were common to fetuses from the treated and control groups.

3. Skeletal examination

The mid-dose fetus with multiple external and visceral defects also had multiple skeletal defects mainly of the skull and vertebral column. One control fetus had fused mandibles and multiple variations in the vertebrae and ribs. Two low-dose fetuses from separate litters had an abnormal and incomplete right tympanic annulus. In the control, low-, mid-, and high-dose groups the number of fetuses(litters) with a partially ossified 5th sternebra was 63(20), 72(18), 81(18), and 106(22), respectively. The proportion of fetuses affected with partially ossified 5th sternebra was significantly ($p \leq 0.05$ or 0.01; 26.5-35.7%) increased for all treated groups as compared with that of the control group (18.2%). In the historical control data provided with the report, the proportion of fetuses affected with partially ossified 5th sternebra was 21.7-51.7%. The incidence of litters, however, showing this variation was not statistically significant.

Skeletal variations common to fetuses from the treated and control groups included incompletely ossified or not ossified vertebrae, sternebrae, odontoid, and skull. Statistical significance in these ossification centers was not seen.

Mean *manus* scores per litter for the control, low-, mid-, and high-dose groups were 3.68, 3.71, 3.76, and 3.85 ($p \leq 0.05$), This slight reduction in ossification was reflected in a shift towards an increase in the mean scores. Mean *pes* scores were 4.09, 4.07, 4.11, and 4.11, respectively and showed no biological or toxicological importance.

TABLE 4: Summary of fetal malformations/variations [No. fetuses (no. litters) affected]*				
Observation	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
Total examined	346 (24)	272 (22)	273 (22)	298 (23)
Major external/visceral defects	1 (1)	0 (0)	3 (3)	1 (1)
Minor external/visceral defects	7 (4)	4 (4)	5 (3)	0 (0)
Major skeletal defects	1 (1)	3 (3)	1 (1)	0 (0)
Minor skeletal defects	92 (24)	78 (21)	81 (21)	69 (22)

*Data taken from Table 10, pp. 42-43, MRID 44364920.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that 1,2-Benzisothiazolin-3-one, 2-butyl- resulted in maternal toxicity at doses of 100 and 300 mg/kg/day as evidenced by ulceration of the stomach. A low incidence of abnormal respiratory noise in high-dose animals may also have been treatment-related. No developmental toxicity was observed. The NOAEL for maternal toxicity was 30 mg/kg/day and the NOAEL for developmental toxicity was 300 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Intercurrent deaths of three animals were confirmed as gavage error, and, therefore considered to be incidental to treatment with the test article. Abnormal respiratory sounds in several high-dose animals are considered treatment-related but may have caused by aspiration of the irritating test article. Gross necropsy was negative in these animals.

Body weight gain was reduced in the high-dose dams during the treatment interval, although the magnitude of lower absolute body weights of the high-dose dams was not biologically significant. However, statistical significance was attained when body weight data were analyzed based on GD 7 body weight which indicated that the effect was on body weight gain. Decreased food consumption corresponded somewhat to the reduced body weight gains by the high-dose animals during the treatment interval. However, the main cause of lower body weight gain by the high-dose dams was probably lower gravid uterine weights.

At necropsy a dose-related increased incidence of ulceration of the stomach [1 (low), 5 (mid), and 16 (high) was observed in all treated groups] vs. a 0 incidence in the

Control. The single incidence of ulceration in the low-dose group was not considered sufficient for setting a LOAEL, for the ulceration may have been due to local irritation by the test article. This phenomena was, likewise, seen in the 90 day feeding study in rats (MRID 444030-01) administered 0, 40, 200 and 2000 ppm where 15 to 20% of the animals of both sexes administered 2000 ppm [♂: 149 mg/kg/day, ♀: 162 mg/kg/day] showed ulcerative lesions in the non-glandular stomach. The lower dose levels and the Control did not show these lesions. The authors considered these lesions to be indicative of irritation of the stomach by the test compound.

Therefore, the maternal toxicity LOAEL for 1,2-Benzisothiazolin-3-one, 2-butyl- in rats is 100 mg/kg/day based on ulcerated areas of the stomach. The maternal toxicity NOAEL is 30 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Intrauterine survival was not affected by the test article. Although slight increases in pre- and post-implantation losses and the percent of early resorptions were observed for all treated groups as compared with the control group, no dose-related trends were observed. Differences in these values by the treated groups are probably due to low values for the control group. All values for the treated groups were within the ranges for the historical data provided. However, the control group values were either at the lower end or below the range of historical data for these parameters.

b. Developmental variations

Maternal treatment with the test article did not have any effect on growth or development of the fetuses. Although the *manus* score for the high-dose group was slightly greater than that of the controls, no other findings suggested an overall reduction in ossification rates of the treated fetuses with the exception of the incidence of fetus showing partially ossified 5th sternebra in the treated groups. However, since the proportion of fetuses with partially ossified 5th sternebra was within the range of historical control, this finding is not considered an adverse effect. Moreover, the litter is used, by the Agency, as the baseline, rather than the fetus, when comparing possible developmental effects. Since no statistical significance was noted in the litter incidence exhibiting partially ossified 5th sternebra vs. the control, this finding is considered of little toxicological importance.

Variations common to the rat fetus were observed equally in the treated and control groups.

d. Malformations

No dose- or treatment-related increased incidence of any malformation was observed.

Therefore, the developmental toxicity NOAEL for 1,2-Benzisothiazolin-3-one, 2-butyl- in rats is ≥300 mg/kg/day [HDT] and the developmental toxicity LOAEL is not identified.

C. STUDY DEFICIENCIES

No deficiencies were identified that would compromise the integrity of this study.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3)] in rats.

EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist,
Team 1, RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Jonathan Chen, Ph.D., Toxicologist,
Team 3, RASSB/Antimicrobials Division (7510C)

S. L. Malish
Jonathan Chen 11/08/01

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding - Rat [OPPTS 870.3100 (§82-1)]

DP BARCODE: D270038 , D270634 SUBMISSION CODE: S587122
P.C. CODE: 098951 CASE: 062095

TEST MATERIAL (PURITY): 1,2-Benzisothiazolin-3-one, 2-butyl (95.5% a.i.)

SYNONYMS: Substance S123386, Vanquish 100 Antimicrobial

CITATION: Rattray, N. J. (1997) Substance S123386: 90 day feeding study in rats (Dolphin Fungicide). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, SK10 UTJ, Report No. CTL/P/5280, February 12, 1997. MRID 44403001. Unpublished.

SPONSOR: Zeneca Biocides (Aveica, Inc.)

EXECUTIVE SUMMARY: In a subchronic oral toxicity study [444030-01], 1,2-benziso-thiazolin-3-one, 2-butyl [corrected for 95.5% a.i. purity] was administered to 20 Wistar-derived Sprague Dawley (Alpk:APfSD) rats/sex/dose each in the feed at concentrations of 0, 40, 200, and 2000 ppm for males and females for 90 days. The mean estimated compound intake in males was 0, 3.1, 15.3, or 149.2 mg/kg/day, respectively, and in females was 0, 3.4, 16.6, or 162.4 mg/kg/day, respectively.

Three males died prior to scheduled termination, one control and two 200 ppm males, but the deaths were not considered treatment-related. No clinical signs were observed. There were no treatment-related neurotoxic effects or effects on motor activity. There were no treatment-related effects of toxicological significance on body weight, food consumption, or food efficiency. Transient depression of body weight and food consumption in the 2000 ppm males and females were attributed to decreased feed palatability and produced an overall body weight gain decrease [12%] at at 2000 ppm. There were no treatment-related effects on ophthalmologic or hematologic endpoints nor were there gross pathologic or organ weight changes.

Histopathologic changes indicative of irritative effects on the non-glandular stomach were observed in 20% each of the 2000 ppm male and female animals. These consisted of minimal to slight submucosal inflammation with or without epithelial hyperplasia or erosion; one male had a slight ulcer. No animals in either of the lower dose groups or the control groups showed such changes. These changes were considered treatment-related and adverse.

Under the conditions of this study, the subchronic toxicity LOAEL is 2000 ppm (males: 149.2 mg/kg/day; females: 162.4 mg/kg/day) based on submucosal inflammation of the stomach. The NOAEL for male and female rats is 200 ppm (males: 15.3 mg/kg/day; females: 16.6 mg/kg/day).

This subchronic oral toxicity study in rats is classified as **Acceptable/Guideline** and satisfies the [OPPTS: 870.3100 (§82-1)] Subdivision F guideline requirements.

COMPLIANCE; Signed and dated GLP, Data Confidentiality, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 1,2-Benzisothiazolin-3-one, 2-butyl

   Description: dark brown liquid
   Lot/Batch #: Blend JM5420/80
   Purity: 95.5% a.i. w/w
   Stability of compound: the test substance was used within the expiration date and stability was confirmed by reanalysis after the in-life phase of the study was ended

2. Vehicle and/or positive control: None

3. Test animals

   Species: rat
   Strain: Alpk:APfSD (Wistar-derived)
   Age and weight at study initiation: 35-42 d; males: 165.3-169.1 g; females: 136.4 - 138.4 g
   Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: Animals were housed in groups of five, cage type not specified
   Diet: Animals were fed CT1 diet, *ad libitum*
   Water: Drinking water was provided, *ad libitum*
   Environmental conditions:
   Temperature: 21 °C ± 2 °C
   Humidity: 55% ± 15%
   Air changes: At least 15 changes/hour
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: ~10-13 days

B. STUDY DESIGN

1. In life dates

   Start: June 10, 1996 ; end: September 12, 1996

2. Animal assignment

Animals were assigned to treatment groups using computer-based randomization (Table 1).

TABLE 1: Study design

Test group	Concentration in diet (ppm)	Dose to males (mg/kg/day)	Dose to females (mg/kg/day)	Number of animals/sex/dose
1	0	0	0	20
2	40	3.1	3.4	20
3	200	15.3	16.6	20
4	2000	149.2	162.4	20

Data taken from pp. 17, 24, MRID 444030-01.

3. Dose selection rationale

Doses were based on the results of a 28-day range-finding study for which no results were presented.

4. Diet preparation and analysis

The experimental diets were made in 55 kg batches by preparing 1 kg premixes using appropriate amounts of the test substance, corrected for 95.5% purity. Each premix was thoroughly mixed with 54 kg of diet. The homogeneity of the test substance was determined by analyzing samples from the low and high dose levels at the top, middle, and bottom of the mixer. The stability of the substance was determined in the 40 ppm mixture on a sample held for 7 days at room temperature and on a sample stored for 42 days at -20°C. Stability was also determined at 2500 ppm in a previous study on a sample held at room temperature for 42 days and also one held for 42 days at -20°C. Concentration analyses were performed on samples taken at two intervals in the study from all dietary levels including controls.

Results-

Homogeneity analysis: For the nominal concentration of 40 ppm, duplicate samples were taken from the top, middle, and bottom; the overall mean concentration was 38.9 ppm. For the 2000 ppm nominal concentration, 4 samples each were taken from the top, middle and bottom; the overall mean concentration was 2040 ppm.

Stability analysis: The 40 ppm sample stored at room temperature was analyzed on days 0, 3, and 7; at day 7, the mean concentration was 92.8% of the initial concentration. The 2500 ppm sample stored at room temperature was analyzed in duplicate on days 0, 5, 12, 20, 28, and 42. The lowest mean value was found at 28 days and was 94.3 % of initial concentration. The 40 ppm sample stored at -20°C was analyzed in duplicate or triplicate on days 0, 7, 21, and 42. The value at 42 days was 95.2% of mean initial concentration. The 2500 ppm nominal concentration sample from a previous study held at -20°C was sampled in duplicate on days 0, 5, 12, 20, 28, and 42. The mean concentration at 42 days was 105.9% of the initial concentration; at 20 days it was the least, 99.3%.

Concentration analysis: Samples were taken from the diets prepared on June 3 and on August 12. They were analyzed in duplicate or in one case quadruplicate. The mean concentrations were all within 10% of the nominal concentration.

Dietary analyses indicated that the test substance was stable in the diets, the homogeneity was satisfactory, and that the doses to the animals were within acceptable limits.

5. Statistics

All data were evaluated by analysis of variance or covariance using the GLM procedure in SAS (1989).

C. METHODS

1. Observations

Animals were examined prior to the beginning of the study and daily thereafter for clinical condition and behavior. Detailed observations were recorded at each weighing. Rats that were moribund were euthanized and a post mortem assessment performed; dead rats were examined as soon as possible after death.

2. Body weight

The body weight of each rat was recorded on day 1 prior to the start of feeding experimental diets and weekly thereafter until study termination.

3. Food consumption and compound intake

Food consumption was recorded throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilization value per cage was calculated as the body weight gained by the rats in the cage per 100 g of food eaten.

4. Ophthalmoscopic examination

All animals were examined pre-experimentally and the control and high-dose animals were examined during the week prior to termination by indirect ophthalmoscope. Examination was performed after instillation of 0.5%v/v Tropicamide into the eyes for pupil dilation.

5. Blood was collected from the tail vein into tubes containing EDTA as an anticoagulant for hematology and lithium heparin for clinical analysis from 10 designated animals during the week prior to feeding the experimental diets and on days 29 and 57. Blood for the clotting measurements was collected using trisodium citrate as an anticoagulant. All animals were bled by cardiac puncture prior to termination. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Red cell distribution width
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

*Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

The CHECKED (X) parameters were measured:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES ▪	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein Electrophoresis
X	Creatine phosphokinase	X	Albumin/globulin ratio
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines
▪ More than 2 hepatic enzymes should be measured.

6. Urinalysis*

The CHECKED (X) parameters were examined. Individual urine samples were collected from designated rats over a 16 to 18-hour period of food and water deprivation prior to taking blood samples on days -1 or -3, 29/30, 57/58, and during the week prior to termination.

X	Appearance (color)	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrate
X	Protein	X	Urobilinogen

7. Functional observational battery (FOB)

An FOB evaluation was performed on 10 rats per sex per group in weeks -1, 5, 9, and 13 in order to assess the neurotoxic potential of the test substance. Detailed clinical assessments (in which each animal was removed from its cage and physically examined for changes in general health status) and quantitative assessment of landing foot splay, muscle weakness (fore- and hindlimb grip strength) and sensory perception were performed by one observer who was 'blind' with respect to the treatment groups. The observations included, but were not limited to, the following list of measures:

1. Assessment of signs of autonomic function, e.g., lachrymation, salivation, piloerection, exophthalmos, urinary incontinence, diarrhea, pupillary response to light, and ptosis.

2. Description of the incidence and severity of any convulsions, tremors, or abnormal motor movements, both in the home cage and the standard arena.

3. Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypes), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that might facilitate interpretation of the data.

4. In addition, the evaluation included the following:

a. Handling observations

Ranking the subject's reactivity to general stimuli such as removal from the cage or handling.

b. Open field observations

Ranking the subject's arousal level or state of alertness during observation of the unperturbed subject in the standard arena.

Description and incidence of posture and gait abnormalities observed in the home cage and in the standard arena.

c. Sensorimotor tests/reflexes

Assessment of audition by response to a sudden sound. The tail flick test was also used to assess sensory perception.

8. Motor activity

Using the designated animals, locomotor activity was monitored by an automated activity recording apparatus in a separate room to minimize disturbances. The designated animals were tested at weeks -1, 5, 9, and 13 in observation periods divided into 10 scans of 5 minutes each. Treatment groups were counterbalanced

across test times and across devices; each animal was tested in the same activity monitor at approximately the same time of day.

9. Sacrifice and pathology

After 90 days of treatment, all surviving animals were killed by exsanguination under terminal anesthesia and necropsied. The CHECKED (X) tissues in the table below were collected from the controls and top dose groups (and any animals found dead), processed, and examined microscopically. In addition, stomachs from all rats in groups 2 and 3 (40 and 200 ppm) were collected and examined microscopically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue (oral cavity)	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*+	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord
X	Stomach*	X	Lymph nodes*		(3 levels)*
X	Duodenum*	XX	Spleen*+	X	Pituitary*
X	Jejunum*	XX	Thymus*+	X	Eyes (retina, optic nerve)*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	XX	Adrenal gland*+
X	Rectum*	X	Urinary Bladder*		Lacrimal gland
XX	Liver*+	XX	Testes*+	X	Mammary gland*
	Gall bladder*	X	Epididymides*+	X	Parathyroids*
X	Pancreas*	X	Prostate*	XX	Thyroids*
		X	Seminal vesicle*	X	Harderian gland
	RESPIRATORY	X	Ovaries and oviducts*+	X	Zymbal's gland
X	Trachea*	X	Uterus*+		
X	Lung*	X	Vagina		OTHER
X	Nose*	X	Cervix	X	Bone
	Pharynx*			X	Skeletal muscle
	Larynx*				Ears
				X	Skin*
				X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity

Those clinical changes seen in animals surviving to scheduled termination were of types and incidences typical of rats of this strain and weight range and were considered unrelated to treatment.

2. Mortality

Three deaths in male rats were observed in the course of the study; one control animal was found dead in week 6 and one 200 ppm male was found dead in each of weeks 2 and 13. In no case were clinical changes observed in these animals prior to death. All females survived to termination.

B. BODY WEIGHT AND WEIGHT GAIN

Body weights in males and females given 2000 ppm test substance were 18 and 10% below control values, respectively, after one week of exposure. The difference from controls decreased to about 6% in both sexes and stayed there throughout the remainder of the study. Body weight gains were below control values in the 2000 ppm groups after one week of exposure by 71% and 63 % in males and females, respectively. By study end, weight gains were decreased by 4.0% and 12% in males and females, respectively. Body weight and body weight gain data for selected weeks are presented in Table 2.

TABLE 2: Selected body weights and body weight gains of male and female rats fed 1,2-Benzisothiazolin-3-One, 2-Butyl for 90 days

Week	Body weight, g; SD, N, adjusted mean[a] (g)							
	Male treatment groups, ppm				Female treatment groups, ppm			
	0	40	200	2000	0	40	200	2000
1 (initial)	168.9 16.5 20	169.1 15.9 20	165.3 14.3 20	168.4 12.9 20	138.4 10.1 20	187.3 8.3 20	136.4 8.7 20	136.7 9.5 20
2	225.0 17.1 20 223.9	225.7 17.9 20 224.3	221.8 13.6 20 224.9	184.5 16.2 20 184.0** (-18)[b]	167.9 8.9 20 166.6	167.4 9.4 20 167.2	166.1 8.8 20 167.0	148.6 11.2 19 149.1** (-10)
4	321.7 23.9 20 319.9	323.4 23.7 20 321.2	318.9 15.0 19 322.8	295.8 20.3 20 294.8** (-7.8)	204.2 12.8 20 203.1	202.2 14.8 20 202.1	201.7 15.6 20 202.4	191.7 12.7 20 192.1** (-5.4)
8	428.8 39.4 19 425.8	433.7 33.8 20 430.6	428.4 21.4 19 432.8	408.7 34.1 20 407.2* (-4.4)	247.8 15.5 20 246.4	245.7 17.2 19 246.3	246.7 20.9 20 247.4	232.5 14.4 20 232.9** (-5.5)
14	541.4 51.1 19 537.6	549.2 37.5 20 545.4	541.2 27.6 18 546.9	508.8 44.4 20 507.1** (-5.7)	279.0 23.1 20 277.7	277.3 18.7 20 277.2	277.7 23.3 20 278.5	260.7 19.4 20 261.2** (-5.9)
Body weight gain[c] (g)								
2	56.0	56.6	56.5	16.2 (-71)	29.5	30.1	29.8	10.8 (-63)
Overall	354	380	376	340 (4.0)	141	140	141	124 (-12)

Data taken from Table 7, pp. 60-63, and Appendix 4, pp. 880-887, MRID 44403001.

**Indicates value different from control mean, $p<0.01$.

*Indicates value different from control mean, $p<0.05$.

[A]Means adjusted for body weight.

[B]Numbers in parentheses are percent difference from control value, calculated by reviewer.

[C]Group mean body weight gains calculated from individual weight gain data by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption was significantly decreased in the 2000 ppm male and female groups (by 39% and 29%, respectively, $p<0.01$) after 1 week of treatment (Table 3). The consumption in these groups remained lower than controls throughout the treatment period, being about 9% lower than controls in both sexes by the end of the study. Food consumption in all other treatment groups was equivalent to controls throughout the study.

TABLE 3: Selected values for food consumption by male and female rats fed 1,2-Benzisothiazolin-3-One, 2-Butyl for 90 days

Consumption, g/rat/day, N = 4								
Week	Male treatment groups, ppm				Female Treatment Groups, ppm			
	0	40	200	2000	0	40	200	2000
1	25.0 SD 1.3	25.2 0.7	24.6 0.9	15.2** 0.9 (-39)[a]	18.9 0.8	19.0 0.3	18.5 0.2	13.4** 0.5 (-29)
6	28.7 SD 1.7	29.6 0.3	29.1 1.1	27.9 1.6	20.4 0.8	20.4 0.7	20.4 0.8	18.4** 0.6 (-9.8)
9	27.6 SD 1.4	27.8 2.2	28.1 2.7	25.4* 1.7(-8.0)	18.5 1.8	18.8 1.2	18.2 1.7	16.5* 2.0 (-11)
13	27.7 SD 1.2	28.1 2.2	27.9 1.5	25.2* 1.4 (-9.0)	17.5 1.7	17.4 0.9	17.3 0.7	15.9* 1.3 (-9.1)

Data taken from Table 8, pp. 64-67, MRID 44403001.
**Significantly different from control at 1% level.
*Significantly different from control at 5% level.
[a]Numbers in parentheses are percent decrease relative to untreated controls, calculated by the reviewer.

2. Compound consumption

Test compound intake was estimated weekly based on nominal diet concentration, food consumption, and body weight. The 13-week averages are given in Table 1.

3. Food efficiency

Food efficiency in treated animals of all groups was equivalent to that of the control animals throughout the treatment period.

D. OPHTHALMOSCOPIC EXAMINATION

No changes upon ophthalmoscopic examination were seen that were considered to be treatment related.

E. BLOOD WORK

1. Hematology

There were statistically significant changes in some parameters. These were scattered, mostly minor, and/or showed no dose response pattern. They are not considered biologically or toxicologically significant.

2. Blood clinical chemistry

Plasma cholesterol levels were slightly but statistically increased in the high-dose male group at Week 9 and in the high-dose female group at Weeks 5 and 14 (23% and 17%, respectively) based only on the covariant-adjusted mean. At Week 14, covariant-adjusted mean plasma cholesterol was also slightly elevated in the 40 ppm female group (13%). Covariant-adjusted mean alkaline phosphatase levels were slightly but significantly increased in the high-dose male group at Weeks 5, 9, and 14 (25, 20, and 14%, respectively) and in the 40 and 200 ppm females in Week 5 (18 and 20%, respectively). No clinically or toxicologically significant differences were found.

F. URINALYSIS

No changes in urine parameters were seen that were considered to be treatment related.

G. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

There were no clinical findings that were considered to be treatment-related, nor changes in landing foot splay, time to tail flick, or changes in grip strength. There were no treatment-related changes in open-field activity, responses to handling, or other sensorimotor endpoints.

H. MOTOR ACTIVITY

There were no treatment-related effects on motor activity.

I. SACRIFICE AND PATHOLOGY

1. Organ weight

No biologically meaningful differences in absolute or relative organ weights were observed.

2. Gross pathology

No treatment-related gross findings were observed.

3. Microscopic pathology

In the three animals that died prior to scheduled termination, one 200 ppm male had a fibrosarcoma of the heart. No microscopic findings were seen that established the cause of deaths of a control male or another 200 ppm male.

Changes were observed in the non-glandular stomach region in the 2000 ppm male and female groups that appear to be treatment-related (see Table 4). Three males showed minimal or slight inflammation of the submucosa, and of these, two showed slight focal hyperplasia of the epithelium, and one additionally had minimal erosion. A fourth male had a slight ulcer in the non-glandular stomach region. Four female rats ingesting the highest dose showed either minimal focal inflammation of the submucosa (two) or minimal to slight erosion in the non-glandular region (two). No such changes were seen in either sex in the lower dose groups or control animals. No treatment-related changes were observed in any other organ or tissue in either sex.

TABLE 4: Selected histopathology changes in male and female rats fed 1,2-Benzisothiazolin-3-One, 2-Butyl for 90 days

Stomach, non-glandular	Male treatment groups, ppm			
	0	40	200	2000
Hyperplasia: epithelium (total)	0	0	0	2
Slight	0	0	0	2
Inflammation: submucosa (total)	0	0	0	3
Minimal	0	0	0	1
Slight	0	0	0	2
Ulcer (total)	0	0	0	1
Slight	0	0	0	1
Erosion (total)	0	0	0	1
Minimal	0	0	0	1
Slight	0	0	0	0
Stomach, non-glandular	Female treatment groups, ppm			
	0	40	200	2000
Inflammation: submucosa (total)	0	0	0	2
Minimal	0	0	0	2
Slight	0	0	0	0
Erosion (total)	0	0	0	2
Minimal	0	0	0	1
Slight	0	0	0	1

Data taken from Table 26, p. 199, and Appendix 12, pp. 1292-1369, MRID 44403001.

III. DISCUSSION

A. DISCUSSION

Three deaths in male rats occurred prior to scheduled termination; one 200 ppm male had a fibrosarcoma of the heart. Deaths of a control male and another 200 ppm male could not be explained. None of the deaths were considered treatment-related in the absence of any mortality in the 2000 ppm male group.

Modest but statistically significant decreases in body weight were seen in the 2000 ppm male and female groups coupled with decreases in food consumption which were most pronounced in the first week of test compound ingestion. The depressed body weight stabilized at about 6% less than controls and is considered a result of decreased palatability of the diet as food consumption was markedly reduced in these highest-dose groups (39% and 29% in males and females, respectively) in the first week of exposure, remaining lower than controls and being about 9% less in both sexes at study termination. Body weight gains which were far less than control values (71% and 63% in males and females, respectively) after one week of exposure were depressed by 4.0% and 12% overall for the 2000 ppm males and females, respectively. Food efficiency was not different from controls in either sex. The decreased body weights and body weight gains are not considered biologically or toxicologically significant.

The authors considered the slight increases in covariant-adjusted mean plasma cholesterol levels and alkaline phosphatase activity at the high dose to be indicative of altered liver metabolism, but the reviewer does not consider these changes to be biologically or toxicologically meaningful. The study authors also considered increased adjusted liver weight relative to body weight in the highest-dose females to be supportive of altered liver metabolic activity but the reviewer does not consider this difference to be meaningful in the absence of any liver pathology.

Histopathological changes in the non-glandular region of the stomach indicative of irritation were observed in four rats each of the 2000 ppm male and female groups. They included minimal to slight submucosal inflammation in three males, with slight epithelial hyperplasia and minimal or slight erosion in two of these. In one male a slight ulcer was reported. Taken together, these changes are considered treatment-related and adverse, although statistical significance was not reached for any of the specific effects. It appears that a threshold for irritative effects may have just been reached. The irritative effects did not affect body weight gains in the animals as the mean body weight gains of these individual animals were higher than (males) or equal to (females) the group mean overall body weight gains at this highest dose level. No such changes were observed in any animal at lower doses or in the control groups and no other treatment-related histopathological effects were reported.

Under the conditions of this study, the subchronic toxicity LOAEL is 2000 ppm (males: 149.2 mg/kg/day; females: 162.4 mg/kg/day) based on submucosal inflammation of the stomach. The NOAEL for male and female rats is 200 ppm (males: 15.3 mg/kg/day; females: 16.6 mg/kg/day). It should be noted that the animals could probably have tolerated a higher dose.

B. STUDY DEFICIENCIES

The animals in this study could probably have tolerated a higher dose of the test article; higher dosing may have produced more clearcut effects.

A minor deficiency is that the pharynx and larynx were not examined histopathologically. In addition, the following required organs were not weighed: heart, epidydimides, ovaries, and uterus. The interpretation of results was not affected by these deficiencies.

EPA Reviewer: Steven L. Malish, Ph.D.,
Team 1 RASSB/Antimicrobials Division(7510C)
Secondary Reviewer: Jonathan Chen, Ph.D., Team 3,
RASSB/Antimicrobials Division (7510C)

S.L. Malish 3/15/01
Jonathan Chen 3/23/01

DATA EVALUATION RECORD

STUDY TYPE: *S. Typhimurium* and *Escherichia coli* WP2P and WP2P *uvrA* Reverse Gene Mutation Assays [§ 84-2], OPPTS 870.5265 and 870.5100

DP BARCODE:	D270038	SUBMISSION CODE:	S587122
P.C. CODE:	098951	EPA ID No.:	072674-E

TEST MATERIAL: N-Butyl-1,2-benzisothiazolin-3-one

SYNONYMS: Vanquish 100 Antimicrobial

CITATION: Callander, R.D., Substance S123386: An Evaluation of Mutagenic Potential using *Salmonella typhimurium* and *Escherichia coli*. Central Toxicology Laboratory, Cheshire, UK. Report No. CTL/P4957. Study No. YV384. August 27,1996. MRID 443649-21. Unpublished.

SPONSOR: Zeneca Biocides, Wilmington, DE 19850

EXECUTIVE SUMMARY: In two bacteria reverse gene mutation assays (MRID 443649-21), *Salmonella typhimurium* strains TA98, TA100, TA 1535 and TA1537, and *Escherichia coli* strains WP2P and WP2P uvrA were initially exposed to a water solution of N-Butyl-1,2-benzisothiazolin-3-one (95.5% a.i.) using the standard plate assay at concentrations *of* 20 µg to 1000 µg/plate (+S9) and 5 µg to 200 µg/plate (-S9). The test substance was then retested in all 6 strains over the dose range of 50 to 2,500 µg/plate (+S9) and 10 to 500 µg/plate (-S9); the S9 mix phase of the second assay was conducted using a pre-incubation protocol. The incubation period for each experiment was 3 days at 37° C. Positive and solvent control substances were run concurrently.

N-Butyl-1,2-benzisothiazolin-3-one was cytotoxic at the highest concentrations used in the presence and absence of S9 mix. The

solvent and positive control gave the appropriate results.

N-Butyl-1,2-benzisothiazolin-3-one was not considered a mutagen to *S. typ.* or *E. coli*, with and without S9, when tested up to a predetermined maximum concentration under conditions of the study.

This study is classified as **Acceptable**, and satisfies the requirements for Guideline § 84-2 for detection of gene mutations in bacteria cells.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials

1. Test Material: N-Butyl-1,2-benzisothiazolin-3-one
[6 concentrations/strain, 3 plates/concentration]

Description:	Dark brown liquid
Batch #:	Blend JM5420/80
Purity:	95.5% a.i.
Stability:	Not specified
Solvent used:	Water
Other comments:	The test substance was stored at room temperature in the dark.

2. Control Materials:

(a) Solvent: Water (100 µl), [5 plates]

(b) Positive Controls

Positive Controls[*,+]

Chemical++	Supplier	Solvent	S9/Strain
Acridine Mutagen (ICR191)	Sigma	DMSO	**-S9**; TA1537
2-Aminoanthracene (2AA)	Sigma	DMSO	**+S9**; all strains
Daunomycin HCl (DR)	Sigma	DMSO	**-S9**; TA98
N-Ethyl-N'-nitro-N-nitroguanidine (ENNG)	Sigma	DMSO	**-S9**; WP2P *uvr*A
Mitomycin C (MMC)	Sigma	H_2O	**-S9**; WP2P
Sodium Azide (NaZ)	Sigma	H_2O	**-S9**; TA1535, TA100

*Adapted from MRID 443649-21, p.14.
+3 concentrations/strain, 2 plates/concentations

2. Dosing Preparations

An individual stock emulsion of the test substance at a concentration of 10 mg/ml or 25 mg/ml, respectively, was prepared for each experiment in water and serial dilutions were carried out as required in each case. The positive control substances were prepared as solutions in the solvents noted in the table above.

All test and positive controls were prepared as close to the time of culture treatment as possible and were dosed at a dosing volume of 100 µl/plate (positive controls in the pre-incubation experiment reduced to 20 µl/plate).

3. S9 Mix

The study was conducted with and without S9 mix incorporated in the top-agar. The S9 mix was prepared as required on the day of the experiment as follows:

	Vol/30 ml S9-mix
S9 fraction	3 ml
Sucrose Tris EDTA buffer (S9 buffer)	7 ml
Cofactor solution	20 ml

In tests without metabolic activation, the S9 fraction and cofactor solution were replaced by an equivalent volume of S9 buffer. Both the S9 mix and the S9 buffer were kept on ice until used.

S9 was prepared from the livers of male Sprague Dawley rats dosed once daily(oral gavage) for 3 days with a combined phenobarbital (80 mg/kg) and β-Naphthoflavone (100 mg/kg) corn oil solution. The treated animals were sacrificed on the day following the third dose. A 25% w/v homogenate (S9 fraction) was then prepared.

The cofactor solution was prepared as a single stock solution Na_2HPO_4, KCl, glucose-6-phosphate, NADP (Na salt) and $MgCl_2$ (150:49.5:7.5:6:12 mM) in sterile dilute water and adjusted to a final pH of 7.4.

4. Test Cells

(a) Test organisms:

(i) *S. typhimurium* strains: TA98, TA100, TA1535, TA1537

(ii) *E. coli* strains: WP2P, WP2P *uvr*A

Properly maintained? Y
Checked for appropriate genetic markers? (Crystal violet, ampicillin resistance, DNA repair deficiency) Y

The presence of the *uvrB* deletion (Salmonella) and the *uvrA* (E. Coli) was confirmed by testing the sensitivity of each culture to mitomycin C (10 µl of a 10 µg/ml solution) in the same manner as sensitivity to crystal violet was tested. Damage to DNA caused by mitomycin C is repaired in normal bacteria by the *uvr* excision repair pathway and is thus toxic to strains deficient at either the *uvrA or uvrB* pathway.

B. Methods

Start: 2/6/1996, Completion: 2/16/1996

3. Experimental Design

In two bacteria reverse gene mutation assays (MRID 443649-21), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2P and WP2P *uvrA* in the were exposed to N-Butyl-1,2-benzisothiazolin-3-one (95.5% a.i.), in water, at concentrations *of* 20 µg to 1000 ug/plate with S9 and 10 µg to 500 µg/plate without S9. The test substance was retested in all 6 strains over the dose range of 50 to 2,500 µg per plate with S9 and 10 to 500 µg/plate without S9. The S9 mix phase of the second assay was conducted using a pre-incubation protocol. The incubation period for each experiment was 3 days at 37° C.

(i) Methodology

0.1 ml aliquots of an overnight culture (10-12 hours) of each bacterial strain were dispended by micropipette into the required number of sterile plastic bijou bottles fitted with screw caps and stored at room temperature until needed.

Top agar consisting of 0.6% w/v agar and 0.5 w/w sodium chlorid in deionized water was melted by brief autoclaving and stored at 50° C until required. Prior to testing, the moten top agar was preparted by adding sterile 0.5 mMhistidine/0.5 mM biotin stock solution (10 ml solution:100 agar) for Salmonella work, and by adding sterile trptophan solution (10 ml solution 0.5nM stock :100 agar) for E. Coli work. Separate agars were prepared for each species.

a) Plate incorporation protocol

0.5 ml of S9 mix (or S9 buffer) was then added by dispensing syringe to the number of bijou bottles of one strain required for one concentration, followed by 0.1 ml of the appropriate concentration of the test substance. Finally, 2.0 ml of top agar was then added by syringe to each bijou, the force of addition was sufficient to mix the contents. The resulting mixture was then poured rapidly onto the surface of a prepared Vogel Bonner plate (9 cm diameter vented Petri dish prepared with 25 ml Vogel Bonner minimal medium containing 1.5% agar and 2% glucose) and allowed to gel. Plates were labeled before being incubated, inverted at 37° C for 3 days in the dark.

Following the total incubation period, the plates were examined for the lack of microbial contamination and evidence that the test was valid, i.e., there was a background lawn on the solvent control plates and on the plates (at least) the lower concentrations of test substance, and that the positive controls had responded as expected.

All plates were counted by an automatic colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies. Contaminated plates were not counted.

b) Pre-incubation protocol

The assay procedure was as for the plate incorporated protocol described above, except that:

1) each 2AA positive control dose was added in 0.02 ml with the total volume made up to 0.1 ml with phosphate buffered saline.

2) before adding the top agar, each compound strain group of bijoux were placed on an orbital shaker(at approximately 140 rpm) for 60 minutes (at 37° C).

5. Evaluation of Results

According to the authors, a test was considered acceptable if:

a) the concurrent solvent control data are acceptable.

b) the positive control data show unequivocal positive responses.

Failure of one or more tester strain/S9 combinations does not

invalidate the data for the remainder of a concurrent experiment.

A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met.

a) a statistical significant dose related increase in the mean number of revertant colonies is obtained;

b) a two fold or greater increase in the mean number of revertant colonies is obtained (over that observed for the concurrent solvent control plates) which is statistically significant, is observed at one or more concentrations.

A negative result in a (valid) individual experiment is achieved when:

a) there is no statistically significant dose related increase in the mean number of revertant colonies per plate observed for the test substance and

b) in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x of the concurrent solvent control.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for the strain/S9 combination, then the observed effect must be consistently reproducible.

Statistical signficancw was carried out using the one-tailed Student's t-test. The corresponding probability for each dose level was derived by computer using the appropriate degree of freedom.

Values of $p<0.01$ wre treated as signifiant, with values of $0.01 \leq 0.05$ being indicative of a possible effect.

II. RESULTS

A. N-Butyl-1,2-benzisothiazolin-3-one Groups

N-Butyl-1,2-benzisothiazolin-3-one did not induce mutagenic activity in any of the 6 bacterial strains used either with or without S9 (Tables 1 to 4). N-Butyl-1,2-benzisothiazolin-3-one was considered toxic (significant thinning/loss of the background lawn) at the highest dose or second highest dose tested (Tables 1 to 4).

B. Control Groups

1. Vehicle Control Groups

The vehicle control groups were within the normal ranges experienced in the particular laboratory.

2. Positive Control Groups

Values from the positive control groups were within the normal ranges expected for each bacterial stain and activation condition.

III. DEFICIENCIES

The test in the original report notes that 10 to 500 µg/plate was used with S9, however, Table 2, p. 21, reports that a concentration of 5 to 200 µg/plate was actually used. These differences in concentration would not affect the integrity of the study.

IV. CONCLUSIONS

N-Butyl-1,2-benzisothiazolin-3-one was not considered a mutagen to *S. typ.* or *E. coli* when tested up to a predetermined maximum concentration with or without S9 under conditions of this study.

Appendix

TABLE 1 - TEST DATA FOR EXPERIMENTAL PHASE 1 (+S9)

COMPOUND Y08496/005 SUBSTANCE S123386

STUDY YY3844 TEST 00 TECHNICAL GRADE EX HUDDERSFIELD SPONSOR ZENECA SPEC. BIOCIDES EAST BLEND JM6420/80 ASG REF:9511769 DATE TESTED 06/02/96 DATE COUNTED 09/02/96

COMMENTS BACKGROUND LAWN SPARSE/ABSENT ON 1000ug DOSE PLATES.

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	+S9	1000	14.3	4.9	0.8		12	11	20
		500	18.0	1.0	1.0		17	19	18
		200	14.7	6.0	0.8		21	14	9
		100	14.7	7.5	0.8		19	19	6
		50	14.3	4.2	0.8		19	11	13
		20	17.3	4.7	1.0		21	12	19
TA 1537	+S9	1000	3.3	0.6	0.7		3	3	4
		500	4.3	1.2	0.9		5	5	3
		200	4.3	0.6	0.9		4	4	5
		100	5.3	2.3	1.2		4	8	4
		50	4.7	1.2	1.0		6	4	4
		20	4.0	1.0	0.9		4	5	3
TA 98	+S9	1000	14.7	3.1	0.7		18	12	14
		500	23.0	6.6	1.1		30	17	22
		200	19.3	2.9	0.9		21	21	16
		100	25.7	7.8	1.2		17	32	28
		50	22.0	4.6	1.0		21	27	18
		20	18.3	2.3	0.8		17	17	21
TA 100	+S9	1000	64.3	4.7	0.7		68	66	59
		500	85.0	7.9	0.9		88	91	76
		200	89.3	8.4	1.0		84	86	99
		100	89.0	10.6	1.0		92	98	77
		50	67.0	14.9	0.9		81	76	104
		20	99.0	7.0	1.1		91	102	104
WP2P	+S9	1000	30.3	16.2	0.7		33	45	13
		500	51.3	10.1	1.1		42	60	52
		200	54.0	4.4	1.2	*	57	49	56
		100	46.7	5.1	1.0		41	51	48
		50	47.7	9.1	1.1		49	38	56
		20	52.0	7.2	1.1		60	46	50
WP2P uvrA	+S9	1000	101.3	27.5	0.6		84	87	133
		500	150.0	7.5	1.0		153	159	138
		200	192.3	19.8	1.2	*	196	210	171
		100	187.7	26.4	1.1		159	193	211
		50	179.0	18.3	1.1		199	175	163
		20	152.7	29.3	0.9		145	128	185

Key to Statistical Significance: * 0.01 ≤ P < 0.05, ** P <0.01
One-sided t-Test assumes Test > Control

TABLE 2 - TEST DATA FOR EXPERIMENTAL PHASE 1 (-S9)

COMPOUND YD8496/005 SUBSTANCE S123386

STUDY YY3844 TEST 01 TECHNICAL GRADE EX HUDDERSFIELD SPONSOR ZENECA SPEC. BIOCIDES EAST BLEND JM5420/80 ASG REF:9511769
DATE TESTED 06/02/96
DATE COUNTED 09/02/96

COMMENTS BACKGROUND LAWN ABSENT ON 200ug DOSE PLATES.

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	-S9	200	9.3	1.5	0.7		8	9	11
		100	10.7	3.1	0.8		14	10	8
		50	9.0	4.0	0.7		9	5	13
		20	10.3	5.1	0.8		16	9	6
		10	9.7	0.6	0.7		10	10	9
		5.0	8.3	0.6	0.6		8	9	8
TA 1537	-S9	200	0.7	0.6	0.2		0	1	1
		100	4.0	0.0	1.1		4	4	4
		50	4.3	1.2	1.2		5	3	5
		20	3.3	0.6	0.9		3	3	4
		10	3.7	0.6	1.0		4	4	3
		5.0	4.0	1.0	1.1		4	3	5
TA 98	-S9	200	13.7	5.0	0.7		19	13	9
		100	17.3	3.1	0.9		20	14	18
		50	19.7	2.1	1.0		17	21	21
		20	18.0	3.5	0.9		22	16	16
		10	20.0	2.0	1.1		18	20	22
		5.0	18.7	1.5	1.0		17	20	19
TA 100	-S9	200	61.0	4.4	0.6		56	49	48
		100	86.7	13.2	1.1		75	84	101
		50	90.7	8.1	1.1		82	92	98
		20	85.0	13.7	1.0		82	100	73
		10	94.7	11.5	1.2		85	80	109
		5.0	76.0	6.1	0.9		83	72	73
WP2P	-S9	200	44.0	15.9	0.9		60	56	26
		100	54.3	3.2	1.1		52	58	53
		50	54.3	3.2	1.1		58	52	53
		20	50.7	8.1	1.0		47	45	60
		10	53.7	10.0	1.1		53	64	44
		5.0	46.3	11.7	0.9		59	35	44
WP2P	uvrA-S9	200	68.0	22.6	0.5		83	62	49
		100	87.3	33.3	0.6		126	62	75
		50	132.3	9.5	0.9		129	143	125
		20	122.3	14.7	0.9		139	111	117
		10	147.0	30.4	1.0		162	167	112
		5.0	144.0	27.1	1.0		156	161	113

Key to Statistical Significance: *: 0.01 ≤ P < 0.05, **: P <0.01
One-sided t-Test assumes Test > Control

TABLE 3 - TEST DATA FOR EXPERIMENTAL PHASE 2 (+S9)

COMPOUND Y08496/005 SUBSTANCE S123386

TECHNICAL GRADE EX HUDDERSFIELD BLEND JW5420/80 ASG REF:9511769

STUDY YV3844 TEST 02 SPONSOR ZENECA SPEC. BIOCIDES EAST DATE TESTED 13/02/96

DATE COUNTED 16/02/96

COMMENTS BACKGROUND LAWN ABSENT/SPARSE ON 2500ug DOSE PLATES.

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	+S9	2500.	2.0	1.7	0.2		4	1	1
		1000.	9.0	3.0	0.8		6	12	9
		500.	7.7	1.5	0.7		8	9	6
		200.	12.7	4.2	1.1		8	14	16
		100.	11.0	1.7	1.0		12	12	9
		50.	11.7	1.5	1.0		12	13	10
TA 1537	+S9	2500.	2.0	1.7	0.5		3	3	0
		1000.	4.7	1.2	1.2		4	6	4
		500.	3.0	1.0	0.8		4	2	4
		200.	3.3	0.6	0.8		4	3	4
		100.	5.0	2.6	1.3		4	3	8
		50.	4.7	1.5	1.2		6	5	3
TA 98	+S9	2500.	5.3	4.2	0.3		2	4	10
		1000.	18.0	2.6	1.0		16	17	21
		500.	16.3	0.6	0.9		16	16	16
		200.	19.3	2.1	1.0		20	17	21
		100.	20.0	4.6	1.1		16	20	28
		50.	20.3	3.5	1.1		17	20	24
TA 100	+S9	2500.	64.7	22.1	0.6		88	44	62
		1000.	105.3	18.5	1.0		84	116	116
		500.	100.0	10.6	1.0		112	92	96
		200.	106.3	19.1	1.0		96	128	92
		100.	124.3	13.6	1.2		111	138	124
		50.	103.7	20.0	1.0		124	103	84
WP2P	+S9	2500.	28.7	1.2	0.6		30	28	28
		1000.	43.7	11.5	1.0		57	37	37
		500.	55.7	12.9	1.2		61	65	41
		200.	52.3	10.0	1.1		42	62	53
		100.	56.5	0.7	1.2	**	C	57	56
		50.	55.7	4.2	1.2	**	57	59	51
WP2P uvrA	+S9	2500.	70.0	17.3	0.4		81	79	50
		1000.	136.0	13.5	0.8		150	123	135
		500.	171.3	4.9	1.0		169	168	177
		200.	148.0	41.8	0.9		196	116	136
		100.	160.7	17.9	1.0		168	141	176
		50.	158.7	9.5	1.0		178	169	159

Key to Statistical Significance: *: 0.01 ≤P <0.05, **: P <0.01
One-sided t-Test assumes Test > Control

C = contaminated plate

TABLE 4 - TEST DATA FOR EXPERIMENTAL PHASE 2 (-S9)

COMPOUND Y08496/005 SUBSTANCE S123386

STUDY YV3844 TEST 03 TECHNICAL GRADE EX HUDDERSFIELD SPONSOR ZENECA SPEC. BIOCIDES EAST BLEND JM5420/80 ASG REF:9511769
DATE TESTED 13/02/96
DATE COUNTED 16/02/96

COMMENTS BACKGROUND LAWN ABSENT/SPARSE ON 500/200ug DOSE PLATES.

STRAIN		DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	-S9	500	4.3	4.9	0.5		10	2	1
		200	7.0	4.4	0.8		12	4	5
		100	6.0	1.7	0.7		5	5	8
		50	8.3	3.5	1.0		8	5	12
		20	9.7	0.6	1.1		10	9	10
		10	10.7	4.0	1.2		13	6	13
TA 1537	-S9	500	0.3	0.6	0.1		0	0	1
		200	2.3	0.6	0.9		3	2	2
		100	2.7	1.2	1.0		4	2	3
		50	3.3	1.5	1.3		3	5	5
		20	3.3	1.2	1.3		2	4	4
		10	2.7	0.6	1.0		3	3	2
TA 98	-S9	500	10.0	6.2	0.5		5	8	17
		200	19.3	5.0	1.0		20	14	24
		100	21.3	3.6	1.1		21	18	25
		50	19.0	4.4	1.0		16	17	24
		20	16.3	0.6	0.9		17	16	16
		10	18.3	2.1	1.0		19	20	16
TA 100	-S9	500	80.3	11.0	0.7		68	84	89
		200	103.7	12.0	1.0		112	92	116
		100	98.7	8.5	0.9		96	88	109
		50	89.7	3.4	0.8		90	102	77
		20	97.0	11.5	0.9		98	85	108
		10	102.3	21.4	0.9		91	127	89
WP2P	-S9	500	33.7	9.0	0.9		44	29	28
		200	46.7	4.7	1.3	*	52	43	45
		100	47.3	9.0	1.3	*	37	53	52
		50	44.0	3.6	1.2		43	41	38
		20	41.7	2.4	1.1		39	46	37
		10	39.7	5.5	1.1		40	34	45
WP2P uvrA	-S9	500	56.3	23.1	0.5		68	32	69
		200	107.3	13.3	0.9		104	122	96
		100	104.0	18.5	0.9		119	88	105
		50	109.6	24.7	0.9		127	98	C
		20	129.3	4.0	1.1		125	133	130
		10	113.3	12.0	1.0		125	101	114

Key to Statistical Significance: *: $0.01 \leq P < 0.05$, **: $P < 0.01$
One-sided t-Test assumes Test > Control

C = contaminated plate

Reviewed by: Steven L. Malish, Ph.D., Toxicologist,
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S.L. Malish
Jonathan Chen NOV. 8, 2001

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics (chromosomal aberrations) in human lymphocytes [OPPTS 870.5375 (§84-2)]

DP BARCODE: D270038 SUBMISSION CODE: S587122
P.C. CODE: 09851 CASE NO.: 062095

TEST MATERIAL (PURITY): S123386 (1,2-Benzisothiazolin-3-one, 2-butyl, 95.5% a.i. w/w)

SYNONYMS: Vanquish 100

CITATION: Wildgoose, J. (1996) Substance S123386: *in vitro* cytogenetic assay in human lymphocytes. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, SK10 4TJ. Laboratory Project ID: Report No. CTL/P/5037, Study No. SV0800, July 11, 1996. MRID 44364922. Unpublished.

SPONSOR: Zeneca Biocides, Wilmington, Delaware 19850 (Avecia. Inc.)

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (chromosomal aberrations) (MRID 44364922), human lymphocyte cultures were cultured for 48 hours, exposed to 1,2-Benzisothiazolin-3-one, 2-butyl (Batch No. Blend JM5420/80, 95.5% a.i. w/w) in DMSO for three hours and then incubated for an additional 17 or 41 hours. Exposures were as follows: Cell donor 1 (female): (68 hour sampling time) nonactivated and activated conditions (S9-mix): 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 µg/mL; Cell donor 2 (male): (68 hour sampling time) without S9-mix: 1, 2, 5, 7.5, 10, 12.5 µg/mL; with S9-mix: 2, 5, 7.5, 10, 12.5, 15, 17.5 µg/mL; Cell donor 2: (92 hour sampling time) without S9-mix: 5, 7.5, 10, 12.5 µg/mL; with S9-mix: 10, 12.5, 15, 17.5 µg/mL. The S9-fraction was obtained from phenobarbital/β-naphthoflavone induced male Sprague-Dawley rat liver.

S123386 was tested up to cytotoxic concentrations. Based on mitotic index determinations, cells from donors 1 and 2 treated at test material concentrations of 2.0, 5.0 and 10.0 without S9-mix and 2.0, 7.5 and 15.0 µg/mL with S9-mix were evaluated for chromosomal aberrations at the 68 hour sampling time. Cells from donor 2 treated at 10.0 µg/mL without S9-mix and at 15.0 µg/mL with S9-mix were evaluated for chromosomal aberrations at the 92 hour sampling

time. Statistically significant increases in the mean percentage of cells with aberrations (excluding gaps in all cases) over the solvent control values were seen at the 68 hour sampling time at 10 µg/mL without S9-mix in cultures from both donors, at 15 µg/mL with S9-mix in cultures from both donors and also at 7.5 µg/mL with S9-mix in cultures from donor 1. A statistically significant increase in mean percentage of cells with aberrations was seen at 92 hours without but not with S9-mix. The aberrations seen were predominantly breaks and fragments. The solvent and positive control values were appropriate. **There was evidence of chromosomal aberration induction (clastogenicity) over background.**

This study is classified as Acceptable/Guideline. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5375 (§84-2)] for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description: dark brown liquid
Lot/Batch No.: Blend JM5420/80
Purity: 95.5% a.i. w/w
Stability of compound:
Solvent used: DMSO

2. Control materials

Negative: none
Solvent/final concentration: DMSO / 5 µL/mL
Positive (concentrations/solvent):
Nonactivation: Mitomycin C / 1.0 µg/mL / double deionized water
Activation: Cyclophosphamide / 50 µg/mL / double deionized water

3. Activation: S9 derived from male Sprague-Dawley rats

__ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none			__ hamster
_x_ other			__ other

The inducer was a mixture of 80 mg/kg phenobarbital and 100 mg/kg β-naphthoflavone

S9 mix composition:

A 1:1 mixture of S9-fraction and the following cofactor solution -

150 mM Na_2HPO_4
49.5 mM KCl
7.5 mM glucose-6-phosphate
6 mM NADP (sodium salt)
12 mM $MgCl_2$

Adjusted to final pH of 7.4

4. Test compound concentrations used:

Preliminary cytotoxicity test: (An integral part of the cytogenetic assay)

Cell donor 1: (68 hour sampling time)
Nonactivated and activated conditions: 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 µg/mL

Cell donor 2: (68 hour sampling time)
Nonactivated conditions: 1, 2, 5, 7.5, 10, 12.5 µg/mL
Activated conditions: 2, 5, 7.5, 10, 12.5, 15, 17.5 µg/mL

Cell donor 2: (92 hour sampling time)
Nonactivated conditions: 5, 7.5, 10, 12.5 µg/mL
Activated conditions: 10, 12.5, 15, 17.5 µg/mL

Cytogenetic assay:

Cell donors 1 and 2: (68 hour sampling time)
Nonactivated conditions: 2, 5, 10 µg/mL
Activated conditions: 2, 7.5, 15 µg/mL

Cell donor 2: (92 hour sampling time)
Nonactivated conditions: 10 µg/mL
Activated conditions: 15 µg/mL

5. Test cells

Mammalian cells in culture - human lymphocytes from healthy, non-smoking donors

with previously established low incidence of chromosomal aberrations. Donor 1 was female and donor 2 was male. Cultures were established by adding 0.5 mL of whole blood to RPMI-1640 (Dutch modification) culture medium supplemented with 10% fetal bovine serum, 1.0 UI/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin. The lymphocytes were activated with phytohaemagglutinin (5% v/v).

Properly maintained? Y

Cell line or strain periodically checked for Mycoplasma contamination? not applicable

Cell line or strain periodically checked for karyotype stability? not applicable

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay

Cytotoxicity, as measured by a decrease in mitotic index of test material treated cultures as compared to the solvent control, was an integral part of the cytogenetic assay. Concentrations were selected for cytogenetic evaluation on the basis of the mitotic index determinations.

2. Cytogenetic assay

a. Cell treatment

Cells exposed to test compound, solvent, or positive control for 3 hours (nonactivated), 3 hours (activated)

b. Spindle inhibition

Inhibition used/concentration: Colcemid / 0.4 µg/mL

Administration time: 2 hours (before cell harvest)

c. Cell harvest

Cells exposed to test material, solvent or positive control were harvested 68 or 92 hours after termination of treatment (nonactivated), 68 or 92 hours after termination of treatment (activated)

d. Details of slide preparation

Following the colcemid treatment, cell cultures were centrifuged, the supernatant removed and the cells resuspended in approximately 10 mL of 0.075 M KCl at room temperature and held for 10 minutes. After recentrifuging, the supernatant was removed and the cells were fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise to a volume of approximately 10 mL. The centrifugation / fixation process was repeated several times and slides then made by dropping an aliquot of cell suspension onto clean, moist microscope slides. The slides were air-dried, stained in filtered Giemsa stain (10% Gurr's R66 in buffered (pH 6.8) double deionized water) for 7 minutes, rinsed in water, air-dried and mounted with coverslips in DPX.

e. Metaphase analysis:

No. of cells examined per dose/harvest time: 200 (100 per culture) ; 1000 cells per culture were examined for mitotic index determination.

Scored for structural: Y

Scored for numerical: N

Coded prior to analysis: Y

f. Evaluation criteria

The percentage of cells with aberrations, including and excluding gaps, was determined for each test material dose and for the controls. Cells with only gaps were not included in the statistical evaluations. Aberrations scored were breaks, fragments and minutes, multiple damage, interchanges, rearrangements, gaps and others. Results were considered negative if there was no statistically significant increase in the percentage of cells with aberrations at any test material concentration over the concurrent solvent control value or if a statistically significant increase was seen but the value was within the laboratory's historical solvent control range. Results were considered positive if an increase in the percentage of aberrant cells was seen at one or more concentrations and the value(s) was substantially greater than the laboratory's historical solvent control values (presumably a statistically significant increase although this was not explicitly stated).

g. Statistical analysis

Data were evaluated for statistical significance at $p<0.01$, using the Fisher Exact Probability Test (one sided).

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Cytotoxicity determination was an integral part of the cytogenetic assay and the results were used to select the concentrations of test material to be evaluated for chromosomal aberration induction. Duplicate cultures were used at each dose. A 68 hour sampling time (48 hours in culture, three hours of treatment and 17 hours post-treatment) was used with cells from both donors. In addition, a 92 hour sampling time (48 hours in culture, three hours of treatment and 41 hours post-treatment) was used with cells from donor 2. In the study without S9-mix using a 68 hour sampling time, cells from donor 1 (female) were exposed to nine concentrations of S 123386 ranging from 2 to 25 µg/mL while cells from donor 2 (male) were exposed to five concentrations ranging from 1 to 12.5 µg/mL. Concentrations of 12.5 µg/mL or higher were excessively cytotoxic to cells from both donors and the mitotic indices were not calculated at these concentrations. The mean mitotic index at 10 µg/mL, the highest concentration selected for cytogenetic analysis, was 5.2% and 6.0% for cells from donor 1 and 2, respectively. In the study with S9-mix using a 68 hour sampling time, cells from donor 1 were exposed to nine concentrations of S 123386 ranging from 2 to 25 µg/mL while cells from donor 2 were exposed to seven concentrations ranging from 2 to 17.5 µg/mL. Concentrations of 17.5 µg/mL or higher were excessively cytotoxic to cells from both donors and the mitotic indices were not calculated at these concentrations. The mean mitotic index at 15 µg/mL, the highest concentration selected for cytogenetic analysis, was 4.2% and 5.9% for cells from donor 1 and 2, respectively.

Cells harvested at the 92 hour sampling time were exposed to four concentrations of test material ranging from 5 to 12.5 µg/mL without S9-mix and to four concentrations ranging from 10 to 17.5 µg/mL with S9-mix. Concentrations of 10 and 15 µg/mL were evaluated for chromosomal aberration induction without and with S9-mix, respectively. The respective mean mitotic indices at these two concentrations were 9.3% and 10.2%.

Results of the mitotic index determinations are presented in Appendix Tables 1-3 (MRID 44364922, pp. 21-23).

B. CYTOGENETIC ASSAY

Chromosomal aberration analyses were performed at the 68 hour sampling time on cells that were exposed to test material concentrations of 2.0, 5.0 and 10.0 µg/mL without S9-mix and to concentrations of 2.0, 7.5 and 15.0 µg/mL with S9-mix. Cells from donor 1 and 2 were separately evaluated. Cells from donor 2 were also evaluated at 92 hours following exposure to test material concentrations of 10.0 µg/mL without S9-mix and to15.0 µg/mL with S9-mix. Duplicate cultures were used at each concentration. In the absence of S9-mix, the mean percentage of aberrant cells (excluding gaps) at the 68 hour

sampling time following exposure to 10 µg/mL was 20.00% with cells from donor 1 compared to the solvent control value of 2.00% and 17.00% with cells from donor 2 compared to the solvent control value of 3.50%. These increases were statistically significant at $p < 0.01$. The mean percentage of aberrant cells (excluding gaps) at 2 and 5 µg/mL were not significantly different from the solvent control values. The solvent and positive control values were appropriate. Results from the 68 hour sampling time without S9-mix are summarized in Appendix Table 4 (MRID 44364922, p. 24).

In the presence of S9-mix, the mean percentage of aberrant cells from donor 1, excluding gaps, at the 68 hour sampling time was significantly increased ($p < 0.01$) at 7.5 (4.00%) and 15 µg/mL (8.50%) compared to the solvent control value of 0.00%. A statistically significant increase was seen in cells from donor 2 at 2 µg/mL (4.00%, $p < 0.05$) and at 15 µg/mL (10.00%, $p < 0.01$) compared to the solvent control value of 0.5%. No significant increases in the mean percentage of aberrant cells were seen at the other concentrations tested. The solvent and positive control values were appropriate. Results from the 68 hour sampling time with S9-mix are summarized in Appendix Table 5 (MRID 44364922, p. 25).

Cells from donor 2 were evaluated at the 92 hour sampling time following treatment with 10 µg/mL S123386 without S9-mix and 15 µg/mL with S9-mix. A statistically significant increase in the mean percentage of cells with aberrations was seen without S9-mix (2.5% compared to the solvent control value of 0.00%, $p < 0.05$) but not with S9-mix (3.5% compared to the solvent control value of 1.00%). The solvent and positive control values were appropriate. Results of the 92 hour sampling time are summarized in Appendix Table 6 (MRID 44364922, p. 26).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. S123386 was tested to sufficiently high concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate. Statistically significant increases in the percentage of cells with aberrations (primarily breaks and fragments) were seen both in the presence and absence of S9-mix. S123386 was clastogenic to human lymphocytes *in vitro* as tested in this study.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX
(MRID 44364922)

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE FILE COPY.

TABLE 1 - MITOTIC INDICES IN THE ABSENCE OF METABOLIC ACTIVATION (S9-MIX)

68 HOUR SAMPLING TIME

DONOR 1			DONOR 2		
Treatment	Mitotic Index %	Mean % Mitotic Index	Treatment	Mitotic Index %	Mean % Mitotic Index
Solvent Control (5µl/ml)	10.2 9.8	10.0	Solvent Control (5µl/ml)	11.6 11.7	11.7
Substance S123386 (µg/ml)			Substance S123386 (µg/ml)		
25	a a	-	12.5	b b	-
20	a a	-	10	6.9 5.1	6.0
17.5	b b	-	7.5	e e	-
15	b b	-	5	8.8 11.3	10.1
12.5	c c	-	2	9.8 11.9	10.9
10	4.6[d] 5.7[d]	5.2	1	10.5 9.5	10.0
7.5	e e	-			
5	9.9 10.3	10.1			
2	9.2 11.0	10.1			

a Mitotic index not determined; no metaphases present on slides
b Mitotic index not determined; few metaphases present on slides
c Mitotic index not determined; severe cytotoxic effects on the chromosomal structure; not suitable for chromosomal aberration analysis
d Some cytotoxic effects on the chromosomal structure; suitable for chromosomal aberration analysis
e Mitotic index not determined; not required for concentration selection

TABLE 2 - MITOTIC INDICES IN THE PRESENCE OF METABOLIC ACTIVATION (S9-MIX)

68 HOUR SAMPLING TIME

DONOR 1			DONOR 2		
Treatment	Mitotic Index %	Mean % Mitotic Index	Treatment	Mitotic Index %	Mean % Mitotic Index
Solvent Control (5µl/ml)	9.0 9.8	9.4	Solvent Control (5µl/ml)	6.9 8.8	7.9
Substance S123386 (µg/ml)			Substance S123386 (µg/ml)		
25	a a	-	17.5	b b	-
20	b b	-	15	5.7 6.0	5.9
17.5	c c	-	12.5	e e	-
15	4.4[d] 4.0[d]	4.2	10	e e	-
12.5	7.0 5.9	6.5	7.5	7.3 9.1	8.2
10	e e	-	5	e e	-
7.5	8.9 10.4	9.7	2	10.7 12.3	11.5
5	a a	-			
2	12.7 11.6	12.2			

a Mitotic index not determined; no metaphases present on slides
b Mitotic index not determined; few metaphases present on slides
c Mitotic index not determined; severe cytotoxic effects on the chromosomal structure; not suitable for chromosomal aberration analysis
d Some cytotoxic effects on the chromosomal structure; suitable for chromosomal aberration analysis
e Mitotic index not determined; not required for concentration selection

TABLE 3 - MITOTIC INDICES IN THE ABSENCE AND PRESENCE OF METABOLIC ACTIVATION (S9-MIX)

92 HOUR SAMPLING TIME

DONOR 2 -S9-MIX			DONOR 2 +S9-MIX		
Treatment	Mitotic Index %	Mean % Mitotic Index	Treatment	Mitotic Index %	Mean % Mitotic Index
Solvent Control (5µl/ml)	17.3 15.3	16.3	Solvent Control (5µl/ml)	13.9 16.1	15.0
Substance S123386 (µg/ml)			Substance S123386 (µg/ml)		
12.5	a a	-	17.5	a a	-
10	8.1 10.4	9.3	15	11.9 8.5	10.2
7.5	a a	-	12.5	a a	-
5	a a	-	10	a a	-

a Mitotic index not determined; not required for concentration selection

TABLE 4 - MEAN CHROMOSOMAL ABERRATIONS AND MITOTIC INDICES IN THE ABSENCE OF METABOLIC ACTIVATION (S9-MIX)

68 HOUR SAMPLING TIME

Treatment		Mean % Aberrant Cells Excluding Gaps	Aberrations/ Cell Excluding Gaps	Mean % Mitotic Index
Donor 1				
Solvent Control	5µl/ml	2.00	0.020	10.0
Mitomycin C	1.0µg/ml	42.00**	0.820	3.7Δ
Substance S123386				
	10µg/ml	20.00**	0.290	5.2
	5µg/ml	5.50	0.055	10.1
	2µg/ml	1.50	0.015	10.1
Donor 2				
Solvent Control	5µl/ml	3.50	0.035	11.7
Mitomycin C	1.0µg/ml	35.00**	0.600	2.7Δ
Substance S123386				
	10µg/ml	17.00**	0.205	6.0
	5µg/ml	4.50	0.045	10.1
	2µg/ml	1.50	0.015	10.9

** Statistically significant increase in the percentage of aberrant cells at $p<0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

TABLE 5 - MEAN CHROMOSOMAL ABERRATIONS AND MITOTIC INDICES IN THE PRESENCE OF METABOLIC ACTIVATION (S9-MIX)

68 HOUR SAMPLING TIME

Treatment	Mean % Aberrant Cells Excluding Gaps	Aberrations/ Cell Excluding Gaps	Mean % Mitotic Index
Donor 1			
Solvent Control 5µl/ml	0.00	0.000	9.4
Cyclophosphamide 50µg/ml	36.00**	0.520	5.2Δ
Substance S123386			
15µg/ml	8.50**	0.120	4.2
7.5µg/ml	4.00**	0.040	9.7
2µg/ml	2.00	0.020	12.2
Donor 2			
Solvent Control 5µl/ml	0.50	0.005	7.9
Cyclophosphamide 50µg/ml	32.00**	0.340	6.0Δ
Substance S123386			
15µg/ml	10.00**	0.115	5.9
7.5µg/ml	3.00	0.030	8.2
2µg/ml	4.00*	0.040	11.5

* Statistically significant increase in the percentage of aberrant cells at $p<0.05$ using Fisher's Exact Test (one-sided).

** Statistically significant increase in the percentage of aberrant cells at $p<0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

TABLE 6 - MEAN CHROMOSOMAL ABERRATIONS AND MITOTIC INDICES IN THE PRESENCE OR ABSENCE OF METABOLIC ACTIVATION (S9-MIX)

92 HOUR SAMPLING TIME

Treatment	Mean % Aberrant Cells Excluding Gaps	Aberrations/ Cell Excluding Gaps	Mean % Mitotic Index
Donor 2 +S9-mix			
Solvent Control 5µl/ml	1.00	0.010	15.0
Substance S123386 15µg/ml	3.50	0.035	10.2
Donor 2 -S9-mix			
Solvent Control 5µl/ml	0.00	0.000	16.3
Substance S123386 10µg/ml	2.50*	0.030	9.3

* Statistically significant increase in the percentage of aberrant cells at $p<0.05$ using Fisher's Exact Test (one-sided).

EPA Reviewer: Steven L. Malish, Ph.D.,
Team 1, RASSB/Antimicrobials Division(7510C)
Secondary Reviewer: Jonathan Chen, Ph.D., Team 3,
RASSB/Antimicrobials Division (7510C)

S. J. Malish 5/1/01
Jonathan Chen 5/4/01

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics: Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395[§ 84-2]

DP BARCODE:	D270038	SUBMISSION CODE:	S587122
P.C. CODE:	098951	EPA ID No.:	072674-E

TEST MATERIAL: N-Butyl-1,2-benzisothiazolin-3-one (95.5% a.i.)

SYNONYMS: Vanquish 100 Antimicrobial, Dolphin 100 Antimicrobial

CITATION: Fox, V., Substance S123386: Mouse Bone Marrow Micronucleus Test. Central Toxicology Laboratory, Cheshire, UK. Report No. CTL/P/5171. Study No. SM0801. Sept. 19,1996. MRID 443649-24. Unpublished.

SPONSOR: Zeneca Biocides, Wilmington, DE 19850

EXECUTIVE SUMMARY:

In an *in vivo* bone marrow micronucleus assay (MRID 443649-24), Charles River CD-1 albino mice, were treated by oral gavage with N-Butyl-1,2-benzisothiazolin-3-one (95.5% a.i.) emulsified in corn oil, at the maximum tolerated doses, of 1,250 (♂) or 2,000 (♀) mg/kg. Five males were sacrificed at both 24 and 48 hours while 5 females were sacrificed at both 24 and 48 hours. A cyclophosphamide positive control [5♂,5♀] was run concurrently and sacrificed at 24 hours. A vehicle control (corn oil) was also run concurrently with 5 animals/sex sacrificed at both 24 and 48 hours. Bone marrow cells were harvested at 24 and 48 hours post-treatment from all groups, except the positive control which was harvested only at 24 hours.

The test substance, N-Butyl-1,2-benzisothiazolin-3-one, produced no significant increase in the frequency of bone marrow micronucleated polychromatic erythrocytes at either 24 or 48 hours

after treatment. The positive and vehicle controls induced the appropriate response.

N-Butyl-1,2-benzisothiazolin-3-one is not considered to be a mutagen under conditions of this study.

This study is classified as **Acceptable** (guideline) and satisfies the requirement for FIFRA Test Guideline § 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials

1. Test material: N-Butyl-1,2-benzisothiazolin-3-one

Description:	Dark brown liquid
Batch #:	Blend JM5420/80
Purity:	95.5% a.i.
Stability:	Not specified
Solvent used:	Corn oil
Other comments:	The test substance was stored at room temperature in the dark.

2. Control materials

Vehicle/Route of administration: Corn oil/oral gavage at 10 ml/kg.

Positive/Final dose/Route of administration: Cyclophosphamide dissolved in saline/65 mg/kg/day//oral gavage at 10 ml/kg.

B. Test Animals

Species:	Albino Mouse
Strain:	CD-1
Source:	Charles River Breeding Laboratories, Margate, UK
Groups:	Four (4) groups:(vehicle control, treated (2 dose levels), positive control)
Feed:	CT1 supplied by Special Diets Services
Water:	Freely available by automatic system
Weight:	19.4 to 38.1 gm (male and female at start of dosing, Phase II)
Age:	Young adult 8 to 11 weeks for Phase I and

	5 to 6 weeks for Phase II
Acclimatization:	For Phase II length of time not stated
Housing:	Not specified
Environmental:	Temperature: 19° to 23° C
	Humidity: 40 to 70%
	Photoperiod: 12 hour light/dark cycle
	Air Changes: at least 15 per hour

C. Test Performance

Experimental Phase: Start: 23 January 1996, Completion: 21 February 1996

1. Maximum Tolerated Dose [MTD] (Phase I)

Six (6) groups of from 2 to 5 males and females were administered oral doses of N-Butyl-1,2-benzisothiazolin-3-one ranging from 1250 to 3200 mg/kg/day and observed for 4 days. From the resultant lack of mortalities, the MTD selected was 2000 mg/kg for females and 1250 mg/kg for males. These doses were administered in Phase II of the study.

2. Micronucleus Test (Phase II)

After acclimatization, the mice for Phase II were randomly distributed. Ten male and 10 female animals were weighed and given a single oral dose of corn oil (10 ml/kg) or cyclophosphamide (65 mg/kg). Ten (10) males [1250 mg/kg] and 10 females [2000 mg/kg] were administered the test substance, respectively, emulsified in corn oil. Bone marrow cells were harvested at 24 and 48 hours post-treatment from all groups, except the positive control which was harvested only at 24 hours.

[Note: An additional 2 males were dosed at 1250 mg/kg and 2 females dosed at 2000 mg/kg. These animals were included to replace any animals at these dose levels that were found dead or killed *in extremis* prior to the scheduled termination time. As no study animals died, prior to the scheduled termination time, these additional mice were killed and discarded without smear preparation after the 48 hour kill].

a. Treatment and sampling times

(i) Test compound

Dosing: x once __ twice (24 hr apart)

Sampling (after last dose):

_____ 6 hr _____ 12 hr __x__ 24 hr __x__ 48 hr

(ii) Negative and/or vehicle control

Dosing: _x_ once ___ twice (24 hr apart)

Sampling (after last dose): _____ 6 hr _____ 12 hr
_x_ 24 hr _x_ 48 hr ___ 72 hr

(iii) Positive control

Dosing: _x_ once __ twice (24 hr apart)

(iv) Sampling (after last dose)

_____ 6 hr _____ 12 hr _x_ 24 hr ___48 hr ___ 72 hr

b. Tissues and cells examined

__x__ bone marrow

c. Details of slide preparation

The animals were killed by asphyxiation by halothane inhalation followed by cervical dislocation at 24 and 48 hours. All animals were given a gross examination.

The femurs were quickly dissected out and freed of adherent tissue. Th iliac end of the femur was removed and a fine paint brush was rinsed with saline, wiped to remove the excess and moistened with a solution of albumin (6% w/v in saline). This was then dipped into the marrow canal and two smears were painted on an clean dry microscope slide. This procedure was repeated to give 4 smears of marrow per slide.

The slides were allowed to air dry and stained with polychrome methylene blue and eosin using an automatic staining machine.

d. Slide evaluation

Slides were coded and scored blind, in numerical slide order. Two thousand (2000) polychromatic erythrocytes were examined for the presence of micronuclei for each animal. The slides were examined for evidence of cytotoxicity, which may be manifested by alterations in the ratio of different cell types in the bone marrow. Cytotoxicity was scored by counting the ratio of polychromatic to normochromatic erythrocytes in a sample of 1000 erythrocytes.

e. Criteria for identification of micronuclei

Criteria for identification of micronuclei as described by Schmid (1976).

1. Spherical (or rounded) with well defined edges.

2. Diameters of not less than ≈1/20 of the polychromatic erythrocytes diameter.

3. Dark purple/blue staining.

4. Lie in the same plane as the polychromatic erythrocytes in which it is contained (determined by focusing).

f. Data evaluation

The incidence of micronucleated polychromatic erythrocytes and the percentage of polychromatic erythrocytes in the erythrocyte sample were analyzed, separately for males and females, by the analysis of variance at 24 and 48 hours.

The data for the incidence of polychromatic erythrocytes were transformed using a square root transformation, prior to analysis. The data for the percentage of polychromatic erythrocytes were transformed using the double arcsine transformation of Freeman and Tukey (1950).

Each treatment group mean was compared with the control group mean was compared with the control group mean at the corresponding sampling time using a one-sided Student's test based on the error mean square in the analysis.

g. Data interpretation

The data have been interpreted as follows:

1. No statically significant increase in the incidence of micronucleated polychromatic erythrocytes above the concurrent vehicle control incidences - *Negative*

2. A statically significant increase in the incidence of micronucleated polychromatic erythrocytes above the concurrent vehicle control incidences but which falls within the laboratory's historical control range - *Negative*

3. A statically and biologically significant increase in the incidence of micronucleated polychromatic erythrocytes which is

in excess of a 3 fold increase when compared with both historical and vehicle control incidences - *Positive*

4. An increase of micronucleated polychromatic erythrocytes which is statistically different from the vehicle control incidences but <3 fold in excess of both historical and vehicle control incidences may require further study.

II. RESULTS

Clinical signs observed for animals dosed with the test substance, N-Butyl-1,2-benzisothiazolin-3-one, included ungroomed appearance and piloerection. Clinical signs observed for females dosed at 2000 mg/kg included subdued nature, abdominal respiratory noises, hunched posture and ungroomed appearance. Gross pathology of 3 females showed gas distended intestine. No deaths were noted at either dose level.

No statistical or biologically significant increases in the incidence or percentage of polychromatic erythrocytes between the vehicle control and the test substance, N-Butyl-1,2-benzisothiazolin-3-one, were observed in either males or females at either sampling time investigated (Appendix, Tables 1 and 2).

III. DISCUSSION

Bone marrow toxicity was mentioned in the text, but not denoted on the tables and it is possible that no toxicity occurred. Since a MTD of the test compound was employed, this lack of bone marrow toxicity would not compromise the study.

IV. CONCLUSIONS

The test substance, N-Butyl-1,2-benzisothiazolin-3-one, produced no significant increase in the frequency of bone marrow micronucleated polychromatic erythrocytes at either 24 or 48 hours after treatment. The positive and solvent controls induced the appropriate response.

The test material, N-Butyl-1,2-benzisothiazolin-3-one was not considered a mutagen in this test system.

References

Freeman, M.F. and Tukey, J. W. (1950). Transformation Related to the Angular and the Square Root. Annals. Of Maths Stats. 21, 607.

Schmid W. (1976). The Micronucleus Test for Cytogenic Analysis. In: A Hollaender (ed). Chemical Mutagens: Principles and Methods for Their Detection. Vol 4, Plenum, New York 31-43.

Appendix

TABLE 1 - MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Group	Treatment	Dose	Mean incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	0.60 ± 0.65	0.70 ± 0.67
12	Cyclophosphamide	65mg/kg	21.90 ± 4.70**	
13	Substance S123386	1250mg/kg	1.00 ± 0.61	0.20 ± 0.45

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's t-test (one-sided) on transformed data.

All means based on 5 counts of 2000 PE per animal.

TABLE 1 - MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

Group	Treatment	Dose	Mean incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	0.80 ± 0.67	0.60 ± 0.22
12	Cyclophosphamide	65mg/kg	13.60 ± 2.77**	
14	Substance S123386	2000 mg/kg	0.90 ± 1.47	0.40 ± 0.42

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's t-test (one-sided) on transformed data.

All means based on 5 counts of 2000 PE per animal.

TABLE 2 - MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Group	Treatment	Dose	Mean % of Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	42.2 ± 11.1	38.1 ± 9.0
12	Cyclophosphamide	65mg/kg	41.6 ± 6.7	
13	Substance S123386	1250 mg/kg	35.1 ± 7.7	30.8 ± 13.7

SD = standard deviation.

All means based on 5 counts of 1000 erythrocytes per animal.

TABLE 2 - MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - FEMALES

Group	Treatment	Dose	Mean % of Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	35.5 ± 11.3	31.1 ± 11.4
12	Cyclophosphamide	65mg/kg	50.2 ± 6.3	
14	Substance S123386	2000 mg/kg	40.6 ± 4.6	30.2 ± 6.7

SD = : standard deviation

All means based on 5 counts of 1000 erythrocytes per animal.

Reviewed by: Chris Jiang, Chemist, CJ
Team 1, RASSB/Antimicrobials Division (7510 C)
Secondary Reviewer: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 6/14/01
Team 1, RASSB/Antimicrobials Division (7510 C)

DATA EVALUATION REPORT

STUDY TYPE:	Assessment of Oral Versus Dermal Toxicity (Non guideline study)		
DP BARCODE:	D274986	SUBMISSION CODE:	S587122
PC CODE:	098951	CASE:	062095
TEST MATERIAL:	2-Butyl-1,2-benzisothiazolin-3-one (97.8 % a.i.)		
SYNONYMS:	Dolphin Fungicide, S123386		
CITATION:	Berry, D., S123386: Assessment of Oral Versus Dermal Toxicity; Zeneca Central Toxicology Laboratory, Cheshire, UK; Lab Project ID CTL/DJB/23597, May 23, 1997; MRID 443649-19; Unpublished.		
SPONSOR:	Avecia, Inc, Wilmington, DE		

SUMMARY: In MRID 443649-19, the oral dose route for Dolphin Fungicide (97.8 % a.i.) has been substituted for the dermal route of exposure because of the severe dermal irritation produced.

In an acute oral toxicity study of S123386, 3 out of 5 male and 1 out of 5 female rats died after doses of 5000 mg/kg of S123386, and 1 out of 5 female rats died after doses of 2000 mg/kg; therefore, the oral LD_{50} was estimated to be 4267 mg/kg for males and 4732 mg/kg for females[1].

In an acute dermal toxicity study, irritation was observed but there were no signs of systemic toxicity so the dermal LD_{50} was concluded to be in excess of the limit dose of 2000 mg/kg[2].

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[1]Lees, D. (1996). Substance S123386: Acute Oral Toxicity in Rats. Zeneca Central Toxicology Laboratory. Report No. CTL/P/5067.

[2]Lees, D. (1996). Substance S123386: Acute Dermal Toxicity in the Rat. Zeneca Central Toxicology Laboratory. Report No. CTL/P/4992.

When dermally exposed to S123386 for 4 hours, lesions that marked by sub-epithelial fibrosis and chronic irritation, indicating that the test substance is dermally corrosive[3].

These studies indicate that S123386 although of low acute oral and dermal toxicity is corrosive to the skin which, therefore, limits the doses that can be used in a systemic subchronic toxicity study. Consequently, the oral route was chosen for the toxicological assessment.

In a 90-day feeding study, rats received diets containing 40, 200, or 2000 ppm (equivalent to 3.1 and 3.4; 15.3 and 16.6; and 149.2 and 162.4 mg/kg/day for males and females, respectively). At doses of 2000 ppm, there was a marked reduction in body weight associated with reduced food uptake. At that dose level, signs of toxicity included stomach mucosal irritation, increased liver weight, and increased alkaline phosphatase (ALP) activity. No adverse effects were seen at the other dose levels; therefore, it was concluded that the highest dose with no adverse effects (NOAEL) was 200 ppm (15.3 mg/kg/day for males and 16.6 mg/kg/day for females[4]).

On the basis of the comparison between the acute oral and dermal toxicity data, it would be reasonable to conclude that under the conditions of repeated daily dosing for 90 days, the dermal exposure to S123386 that would be required to produce signs of systemic toxicity would be similar to the oral lowest effect level, that is, 149.2 mg/kg/day assuming the test material was 100 % absorbed through the skin. Therefore, the dermal equivalent of a NOAEL would be similarly achieved through an oral route, about 15 mg/kg/day.

CONCLUSION

The dermal exposure equivalent would probably be similar to the value obtained through the oral route assuming the test material is 100 % absorbed through the skin.

---

[3]Lees, D. (1996). Substance S123386: Skin Irritation in the Rabbit. Zeneca Central Toxicology Laboratory. Report No. CTL/P/4969.

[4]Rattray, NJ. (1997). S123386: 90-day Feeding Study in the Rat. Zeneca Central Toxicology Laboratory. Report No. CTL/P/5280.